O-Acetylserine Sulphydrylase from *Vicia faba*; its Role in Activation of Azide to A Mutagenic Product

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ABSTRACT

In this work, O-acetylserine sulfhydrylase enzyme from *Vicia faba* seedlings was purified to apparent homogeneity by ammonium sulfate, anion-exchange, gel filtration and non-denaturing tube gel electrophoresis. The specific activity of the enzyme recovered from the last step was 535.9 units/mg/min.

The enzyme band eluted from non-denaturing gel electrophoresis was resolved under denaturing conditions (SDS) and showed a single band with a molecular weight of 34,500 dalton. The native molecular weight of the enzyme was determined by gel filtration to be 72,000 dalton. This may indicate that the enzyme consists of two identical subunits, each about 34,500 dalton. Studying the effect of specific inhibitors revealed the presence of a pyridoxal phosphate as a prosthetic group for the enzyme.

The crude and purified enzyme were able to produce a mutagenic product from azide and O-acetylserine on *Salmonella typhimurium* TA 1530. The mutagenic potency of this product is lower than that produced in bacteria.

The kinetic data of the enzyme O-acetylserine sulfhydrylase showed that the Km values for O-acetylserine and sodium sulfide were estimated to be 6.45mM and 4.0mM respectively, while the Ki for the azide was 3.8mM. This strongly suggests that azide and the natural substrate (S--
use the same catalytic site on the enzyme, and the enzyme is inhibited by azide competitively.

The optimum pH of D-acetylserine sulfhydrylase was found to be 7.5 and the enzyme was stable to heating up to 40°C for 10 min. and lost its total activity at 75°C.

Results obtained from double diffusion indicated that the antibody raised against non-denaturing gel electrophoresis enzyme is monospecific. The antibody was also monospecific with the enzyme from *Lens culinaris* and a partial identity has been shown with *Cicer arietinum*, while no identity was observed with enzyme from *Drosophila melanogaster* (larvae and adult) and *Salmonella typhimurium*. Immunotitration proved also the ability of the antibody to inactivate the enzyme from *Vicia faba*. 